

Importance of Selectivity for Reaction Monitoring

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GOAL

To demonstrate the benefit of different selectivity of UPC² compared to LC when used for the analysis of synthetic reaction mixtures.

BACKGROUND

During the development of new chemical entities (NCE), there is a need to analyze reaction mixtures, synthetic intermediates, and isolated compounds. Understanding the purity of the materials used in a reaction provides information that is used to aid reaction rates, inhibit catalyst quenching mechanisms, and provide insight to scalability.

Due to the complexity of reaction mixtures, a single chromatographic method or technique is not sufficient for providing the information scientists require. For example, during the synthesis of a molecule, lack of chromatographic selectivity can lead to poorly separated impurities that often affect the target molecule yield throughout the synthetic pathway.

In terms of high-throughput screening (HTS) using LC, screening strategies including high /low pH have recently been implemented to alter chromatographic selectivity. However, this reaction monitoring process may still be inefficient in separating structurally similar components generated during the synthetic process. It is critical when submitting an NCE to a compound library that identity and purity are assessed, thus, making orthogonal separations strategies important.

Alternative chromatographic selectivity with UPC² allows for robust and accurate characterization of synthetic reaction mixtures.

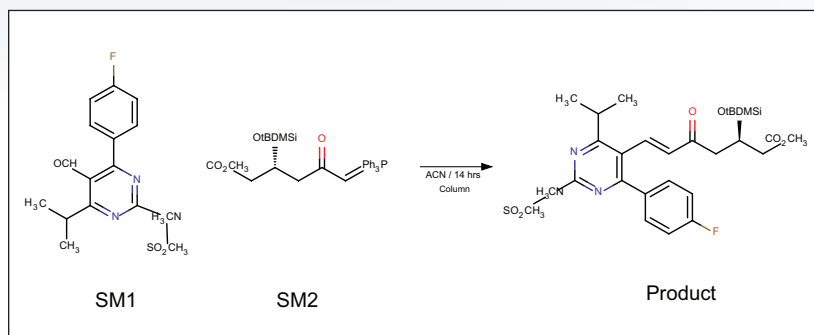


Figure 1. A single reaction step from the rosuvastatin synthesis reaction scheme.

THE SOLUTION

The primary aspects of this investigation evaluate Convergence Chromatography for monitoring the total synthesis of the pharmaceutical drug substance rosuvastatin, an HMG-CoA inhibitor. We used an ACQUITY UPC²™ System configured with an ACQUITY UPC² PDA for UV detection and an ACQUITY® QDa™ Detector for mass detection. For simplicity, we compared the ACQUITY UPC² results from one of the synthetic stages to the results acquired with reversed-phase UPLC® using high/low pH screening. The details of the synthetic step are shown in Figure 1.

Differences in chromatographic selectivity were clearly apparent as shown in Figures 2 and 3. At $T=0$ the ACQUITY UPC² (A), UPLC Low pH (B) and UPLC High pH (C) separations were able to resolve each of starting materials in the reaction mixture (Figure 2). The reaction was monitored using both techniques after several hours at reflux. The UPC² separation resolved both the starting materials and the final product in the mixture (Figure 3). In contrast, neither of the UPLC separations provided enough specificity between the product (PDT) and starting material (SM1). Additionally, the starting material labeled “SM2” appeared quenched in both UPLC separations, but not in the UPC² results.

The monitoring wavelength of 270 nm was suitable for the starting materials; however, 270 nm was not suitable for monitoring the product material during the UPLC analysis. The systems were configured to acquire a 3D PDA MaxPlot data channel ranging from 210 nm to 410 nm. This capability allows for UV spectral analysis and selected wavelength extracted chromatograms. The PDA spectra of the product indicated a lambda max of 295 nm. Therefore, chromatograms were extracted from the MaxPlot data channel to best monitor the reactions without having to change methods prior to next experiments.

Interestingly, the SM2 material is not as easily detected by UPLC at 295 nm. The UV absorbance of the mobile phase interferes with the detection of the SM2, hence appearing as a quenched reaction. Although changing the wavelength was needed to monitor the production of the target molecule, the PDA 3D data collection (MaxPlot) provided the ability to view the results at 270 nm. Deriving a chromatogram at 270 nm combined with the MS data enabled us to determine the presence of SM2 in the clean-up stages of the final material.

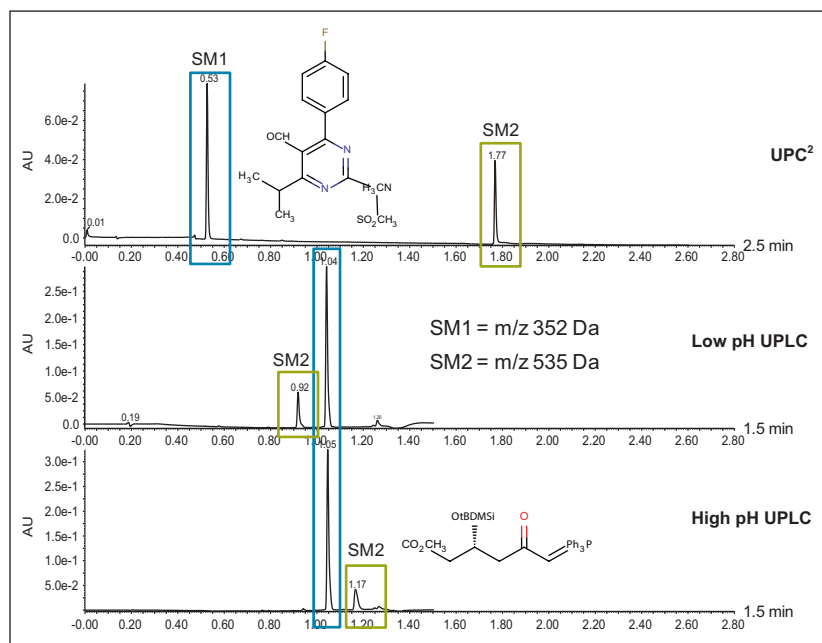


Figure 2. Chromatographic overlays at 270 nm of (A) ACQUITY UPC², (B) UPLC low pH, and (C) UPLC high pH results for the initial time point ($T=0$ min). See figure 1 for SM1 and SM2 structures.

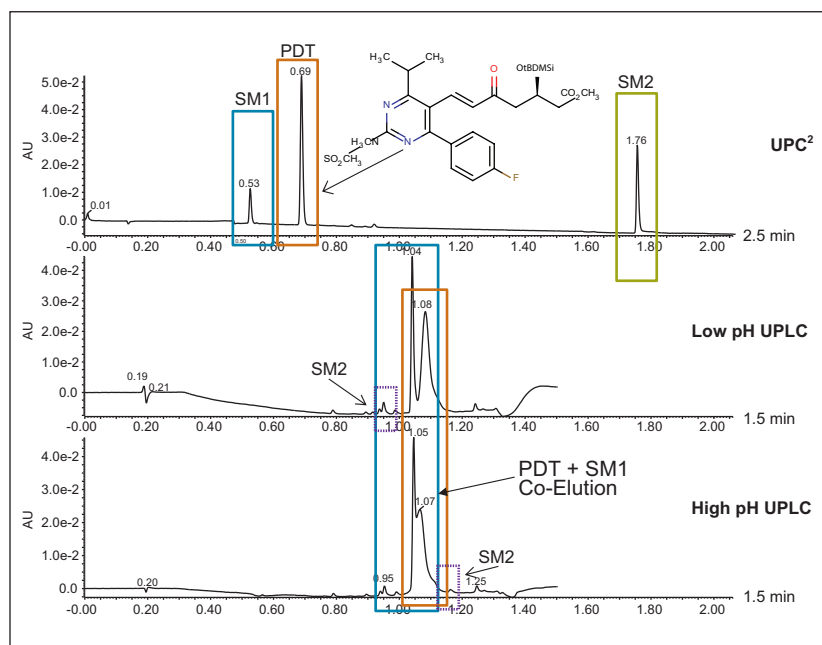


Figure 3. Chromatographic overlays at 298 nm of (A) ACQUITY UPC², (B) UPLC low pH, and (C) UPLC high pH results for time point 5 ($T_5 = 6$ hrs).

SUMMARY

ACQUITY UPC² for the analysis of reaction mixtures provides added selectivity and orthogonality when compared to reversed-phase LC high/low pH screening separations. Extracted wavelengths from the PDA Maxplot provided the ability to monitor reactions at various wavelengths and verify UV spectral profiles without having numerous data files to investigate. In addition, using both MS and PDA detection for either technique provides an accurate and quantitative assessment of the reaction progress and purity.

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